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TOVEY et al	)	Examiner: D. Fitzgerald
Appln. No.: 08/853,292	)	Washington, D.C.
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For: STIMULATION OF HOST DEFENSE MECHANISMS AGAINST VIRAL CHALLENGES	)	**BY HAND-CARRYING**

#29
8/7/01

SUPPLEMENTAL COMMUNICATION

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

The present communication is intended to supplement applicant's amendment of June 27, 2001. At page 9 of the amendment of June 27, 2001, the declaration of Dr. Tovey, which was attached to that amendment, was discussed, as was an additional abstract of Schofield et al, supporting Dr. Tovey's argument that, at the time of the present invention, conventional wisdom held that indirect immunological stimulation of a substance that is not absorbed by the organism in appreciable quantities would not be dose responsive.

Two additional publications, available as of the effective filing date of the present application, have now

been identified which further support our contention that there is no *prima facie* case of obviousness to use doses of interferon greater than that described by Cummins. Attached hereto is Cummins et al, "Oral use of human alpha interferon in cats", J Biol Response Mod 7:513-523 (1988). This publication reports that kittens, infected with fatal feline leukemia virus and given 0.5 U of human IFN-α orally, survived longer (500 ± 41.7 days) than animals given 5.0 U of IFN-α (313 ± 104.8 days) compared to untreated control. In this regard, reference is also made to the Moore et al publication, a copy of which was attached to applicant's amendment of November 17, 2000, showing, particularly in Figure 1, that in horses lower doses are significantly better than control, whereas the higher dose is not significantly better.

Also attached hereto is Brod et al, "Oral administration of IFN-α is superior to subcutaneous administration of IFN-α in the suppression of chronic relapsing experimental autoimmune encephalomyelitis", J Autoimmun 9:11-20 (1996). This paper also reports that oral administration of 10 IU of IFN-α was more effective than 100 or 1000 IU in suppression of chronic relapsing experimental autoimmune encephalomyelitis in rats.

These publications are cumulative to those already cited and discussed in applicant's amendment of June 27, 2001,

and further support the understanding which would have been held by those of ordinary skill in the art at the time the present invention was made. Consideration of the present communication and the attachments thereto in conjunction with applicant's amendment of June 27, 2001, and reconsideration and withdrawal of the rejections of record for the reasons explained in the amendment of June 27, 2001, are hereby earnestly solicited.

Respectfully submitted,

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Oral Use of Human Alpha Interferon in Cats

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Summary: Low doses (0.5 or 5.0 U) of human alpha interferon (HuIFNα) given orally prevented the experimental development of fatal feline leukemia virus (FeLV)-related disease. Twenty-one FeLV-susceptible cats were inoculated with the Rickard strain of FeLV. Cats given oral HuIFNα survived significantly ($p < 0.001$) longer than untreated FeLV-infected cats. Moreover, only 4 of 13 (30.8%) HuIFNα-treated cats developed clinical disease during the course of the study, whereas 100% of the untreated control cats developed fatal FeLV-related disease. Thus, in experimental retroviral disease, heterologous species HuIFNα provided significant clinical benefits. Key Words: Feline leukemia—Human alpha interferon.

The feline leukemia virus (FeLV) is classified in the family Retroviridae (1,2). The FeLV is horizontally transmitted to cats in which it induces lymphoid neoplasia (2–5). In addition to neoplastic disease, FeLV causes degenerative and immunosuppressive disorders such as thymus atrophy, a panleukopenia-like syndrome, and nonregenerative anemia (6,7). The main site of viral replication is the bone marrow, and the clinical disease reflects the type of stem cell infected (1,2,4–6,8).

There are a number of antigens associated with FeLV infection (6,8). The nuclear core group-specific (gs) antigens are used to identify FeLV-infected animals and can be detected in circulating leukocytes by an indirect fluorescent antibody technique (9), and in the plasma or serum by an enzyme-linked immunoabsorbent assay (Feleuk Test, Pitman-Moore Inc., Washington Crossing, NJ, U.S.A.).

FeLV envelope antigens possess determinants that divide FeLV into three major subgroups—A, B, and C. These antigens are major immunogens and will induce neutralizing antibody formation (6,10).

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A third group of antigens associated with FeLV infection are the feline oncornavirus cell membrane antigens (FOCMA) which are induced by the virus but are associated with the cell membrane of the infected cell (6,11).

Upon infection by the virus, one of several host-virus interactions develops in the cat: (a) The cat may develop viral neutralizing antibody and FOCMA antibody, and after a transient viremia, FeLV gs antigen may no longer be detectable in the circulating blood (2,5,6,12,13). (b) A persistent infection may develop in which FeLV gs antigen is continuously detectable in the peripheral blood. The cat eventually develops one of the FeLV-related diseases, which may be directly responsible for the death of the animal. There are usually very low levels of FOCMA antibodies and little or no neutralizing antibody produced (9,12,13). (c) Asymptomatic carriers are gs-antigen positive but develop high titers of FOCMA antibodies. These carriers do not develop the FeLV-related disease but remain viremic and are a source of infection to other cats (12,13).

The horizontal transmission of FeLV has been well documented (2,3). As long as a cat is gs-positive, it is viremic and capable of excreting virus, primarily through the saliva (14). Susceptibility is age-related and in general, kittens are more likely to become persistently infected than adults (5,12).

The prognosis for recovery of clinical cases of FeLV-related disease is poor, with 50% being fatal within 4 weeks and 70% being fatal within 8 weeks after onset of clinical signs (15). Cats respond poorly to treatment for FeLV-related disease (9,16–22). The FeLV-related disease can be attributed principally to opportunistic infections secondary to FeLV-induced immunosuppression. There is growing evidence that FeLV-induced immunosuppression is due primarily to a T helper lymphocyte dysfunction (23–28).

In 1957, a mediator of viral interference was identified and named "interferon" (29). Subsequently, three distinct classes of IFN have been designated α, β, and τ (30–32). In addition to their antiviral properties, IFNs all have immunomodulatory functions (33–38). The α IFNs, produced primarily by leukocytes and macrophages at the site of a viral infection, represent a mixture of IFN isospecies coded by different genes (39–40).

In this report, we describe the use of heterologous species IFN given orally to cats with feline leukemia. The α IFNs were selected for study because they are no longer considered species specific (41). The oral route of administration of IFN has been described in previous reports (42–44).

MATERIALS AND METHODS

Cell Cultures

Bovine fetal kidneys (BFK) obtained at an abattoir were used to prepare BFK cell cultures. WISH (human) cells (Immuno Modulators Labs, Inc., Stafford, TX, U.S.A.) and BFK cells were used in the IFN assay to determine human IFN potency. Growth and maintenance media for cells consisted of Eagle's minimal essential medium prepared in Hanks' balanced salt solution with 10 or 5% bovine fetal serum, respectively, as described (45). Potassium penicillin G and strepto-

mycin sulfate were included in all media. Cultures were incubated at 37°C under CO_2.

Cats

Twenty-one 8-week-old, specific pathogen-free (SPF) cats, six females and 15 males, were used in a challenge study. The 21 SPF cats were from a hysterectomy-derived (46) breeding colony maintained by the Department of Veterinary Pathobiology, the Ohio State University.

Viruses

The Rickard strain (FeLV-R) of subgroup A (47) was used in the challenge study. The viral inoculum was serum of a FeLV-R isolate. Vesicular stomatitis virus, Indiana strain (Dr. Bruce Rosenquist, University of Missouri, Columbia, MO, U.S.A.), was propagated in cultures of BFK cells and was used in IFN assays. Sendai virus (Hazelton Research Products, Inc., Denver, PA, U.S.A.) was used for IFN induction of human leukocytes.

Interferons

The human IFN α (HuIFNα) preparation (Immuno Modulator Labs) was produced by the method of Cantell et al. (48,49). The IFN was an antiviral, proteinaceous compound with the following characteristics: specific activity $\geq 10^6$ U/mg protein; antiviral activity neutralized by partially purified HuIFNα antiserum; molecular mass of 17,000–23,000 daltons; and stability ≥6 months frozen at −20°C.

Interferon Assays

The HuIFNα assays were conducted on WISH and BFK cells using a plaque reduction assay (50). The potency of HuIFNα was calibrated by comparison to the international standard for human IFN (Immuno Modulator Labs).

Experimental Design

Twenty-one cats were given FeLV-R by intravenous inoculation of 0.1 ml of serum. Blood from each inoculated cat was collected weekly for the first 6 weeks and then biweekly or monthly for evaluation of FeLV status. Infected cats were divided into 3 groups. Group 1 (n = 7) did not receive treatment with HuIFNα; group 2 (n = 9) were given 0.5 U HuIFNα, and group 3 (n = 5) received 5.0 U HuIFNα.

The HuIFNα was given orally once daily starting the day of FeLV-R inoculation. Cats were treated for 7 consecutive days every other 7 days until 24–28 weeks (group 2) after FeLV-R inoculation. Treatments to the three surviving cats given 5.0 U of IFN were discontinued at 25–27 weeks after virus inoculation.

Cats that died were necropsied. Gross examinations were done on all

necropsied cats and some tissues were fixed in formalin, methanol, and glutaraldehyde for histologic examinations.

Sampling

For demonstration of FeLV gs antigen in peripheral blood leukocytes, a minor modification (51) of the original indirect immunofluorescence procedure of Hardy et al. (9) was used. The primary reagent was goat anti-FeLV; the antiserum was absorbed extensively with normal feline tissues prior to use (51). Titers for FOCMA antibody titers were determined with FL-74 cells as described (52,53).

Statistical Analysis

Data were subjected to analysis of variance and Chi square techniques. The α level test was $p < 0.05$ for all comparisons.

RESULTS

Prevention of the development of FeLV-related diseases with HuIFNα was achieved in this challenge study. Cats given the lower dosage of 0.5 U of HuIFNα once daily for 7 days, every other 7 days, survived significantly ($p < 0.001$) longer than control cats (Table 1). All seven infected, untreated cats died (at an average of 72.7 days after FeLV inoculation) of a wasting syndrome typical of FeLV-R. One kitten given 0.5 U HuIFNα had a diaphragmatic hernia likely of congenital origin, died 10 days after FeLV-R challenge, and was removed from the study. All of the other eight cats given 0.5 U HuIFNα orally survived well beyond the average survival time (AST) of untreated cats (AST of eight treated $> 500.0 \pm 41.7$ (standard error) days compared with AST of 72.7 ± 13.2 days in untreated cats). Moreover, the frequency of protection with HuIFNα treatment was high in that all eight cats showed increased survival time, and at the time of this writing only two have died (of thymic lymphosarcoma 222 or 458 days after FeLV inoculation). The other six cats given 0.5 U HuIFNα are alive, remain asymptomatic, and have survived an average of 553.3 days.

Two cats given 5.0 U HuIFNα died of a wasting syndrome strain at 35 and 79 days after virus inoculation. At the time of this report, the remaining three cats in

TABLE 1. *Survival and group-specific (gs) antigen status and feline oncornavirus cell membrane antigens (FOCMA) development of cats inoculated with feline leukemia virus and treated with human alpha interferon (HuIFNα)*

Treatment HuIFNα	No. of cats	Average (± SE) days survival	Positive for		Alive[a]
			gs	FOCMA	
None	7	72.7 ± 13.2	7	7	0
0.5	9[b]	500.0 ± 41.7	7	9	6
5.0	5	313.0 ± 104.8	5	5	3

[a] As of July 26, 1988.
[b] One cat died with a diaphragmatic-pericardial hernia of unknown origin and was deleted from all calculations; one other cat died of a thymic lymphosarcoma 222 days after virus inoculation.

this group are alive, remain asymptomatic, and have survived an average of 483.7 days (Table 1). Seven (four given 0.5 U HuIFNα and three given 5.0 U HuIFNα) of the nine surviving cats are viremic. All cats given FeLV-R became positive for FOCMA antibody and the surviving cats remain positive.

DISCUSSION

Interferon as an Immune Modulator

Originally described as an antiviral protein, the IFNs are now recognized to perform numerous functions in the immune system (33–38). The IFNs in general and τ in particular, may induce macrophage Fc receptors, induce receptors for IgG1 in human monocytes and macrophage-like cell lines, induce tumoricidal responses in mouse macrophages, and induce the release of oxygen-derived products from macrophages (33–36). The IFNs increase growth and differentiation of cytotoxic progenitor cells, increase the proportion of effector cells capable of recognizing target cells, and switch on some lytically inactive cells (35,36). The IFNs also play a modulatory role in natural regulation of antibody responses (37). IFN has been detected in the nasal secretions (NS) of animals and is thought to serve as a marker of viral replication. The major type of IFN in the NS of calves and man is IFNα (54,55). Respiratory viral infections of animals and man commonly occur and IFNα is probably the major type of IFN produced. The fate of most NS IFN is ingestion through swallowing.

Because IFNs are proteins and are inactivated by trypsin and other proteolytic enzymes, and because IFN cannot be detected in the blood after oral administration (56–58), IFN administration by the oral route is not practiced in human medicine. Intravenous, intranasal, subcutaneous (s.c.), intramuscular (i.m.), topical, intralesional, and ocular routes of administration are most commonly used. Success in the HuIFNα treatment of hairy cell leukemia by the s.c. or i.m. routes of administration has led to FDA approval in 1986 for two recombinant HuIFNα (rHuIFNα) products (Schering Plough's "Intron" and Hoffmann LaRoche's "Roferon") (59,60). The successful treatment of hairy cell leukemia with high doses of rHuIFNα by parenteral injection has been repeated in some other human viral or neoplastic diseases. But despite some success (by parenteral administration of rHuIFNα) in reducing the duration and severity of rhinovirus infections (61,62); in achieving papillomavirus remissions associated with condyloma acuminatum, epidermodysplasia verruciformis, and warts (63–67); and induction of remission of some other neoplasms (68–71), the response to IFN treatments are often disappointing and accompanied by toxic signs of fever, nausea, pain, and anorexia (72).

Interferon: Cross-Species Use

In vitro, αIFNs are no longer regarded as species-specific proteins (41). The HuIFNα is active in vitro in cells of bovine origin, and BovIFNα is active on cells of human origin (73–80). Although antigenically distinct, the amino acid sequences of some HuIFNα and BovIFNβ are reported to be 60–90% homologous (81,82).

It has been reported that HuIFNα had antiviral activity when tested on virus-infected feline cells in vitro (83). Others reported that HuIFNα (Cantell type) had antiviral activity against FeLV and that HuIFNα was 10 times more active than feline IFN (FeIFN) when tested on feline cell cultures (84). These observations are supported by another report in which HuIFNα protected feline cells against feline viral challenge (85). Seven species of rHuIFNα induced the synthesis of at least five proteins in human fibroblasts but only one such protein was readily detected in feline fibroblasts although rHuIFNα inhibited the replication of three viruses tested (86). No significant clinical benefits were reported in cats given high dosages (10^8 U/kg) of rHuIFNα s.c. after challenge with feline herpesvirus (87). The study reported herein indicates that HuIFNα is active in cats in vivo, and suggests that the oral route of administration is effective.

Interferon and Feline Leukemia

Feline interferon, produced in a feline kidney cell line (CrFK) by Newcastle virus induction, inhibited FeLV in cell culture (88). Feline cells in culture pretreated with FeIFNα or β, and then infected with FeLV, produced high levels of FeLV proteins, but little infectious virus. FeIFNα, but not FeIFNβ, had antiviral activity on canine cells. Acid labile, as well as acid stable, FeIFNα has been described (89).

Supernatants from cultures of normal feline lymphocytes stimulated with *Staphylococcus aureus* protein A (SAP-A) showed antiviral activity, characterized as a τ-like IFN (90). With the addition of inactivated FeLV, less IFN was produced. The reduction in IFN production was not attributable to lowered lymphocyte viability or reduced mitogenic properties of SAP-A and appeared to be a direct retroviral effect (90).

BovIFNβ and HuIFNα administered orally have been previously reported to show some benefit in the treatment of FeLV-related anemia (42,91).

Feline Leukemia Treatment

Cotter reported on 100 cases of anemia associated with FeLV infection. Forty-nine cats failed to respond to various treatments and were euthanized within 2 weeks of diagnosis. Twelve were lost to follow-up and 39 were treated and monitored for 2 weeks to 7 years; median survival time of the latter 39 cats was 4 months. Only 8 of 88 cats (9%) had a return to normal blood counts (16). Ten cats positive for FeLV with clinical signs of leukemia (fever, stomatitis, abscess, vomiting, respiratory signs, iritis and hyphema, transudate, lymphadenopathy, and/or abnormal hematology) all died (some by euthanasia) within 6 months despite blood transfusions, antibiotics, chemotherapy, or steroid treatment (21).

In a number of smaller studies, cats with various FeLV-related diseases, including myelogenous leukemia (17), reticuloendotheliosis (18), monocytic leukemia (19), and myelomonocytic leukemia (20), failed to show significant response to chemotherapy. Only four of 15 leukemic cats treated with chemotherapy achieved remission for 1–24 months (median 7 months) (22). Eighty percent of

persistently chronic viremic cats die within 2 1/2–3 1/2 years, as compared with 10% of uninfected cats of similar ages that die within that period (92). Untreated cats with thymic lymphosarcoma (LSA) positive for FeLV usually die in less than a month after presentation. Loar (22) has reviewed the management of LSA (70% of which are associated with FeLV) and generally reported short-term (only a few months) survival.

Cats seropositive for FeLV and diagnosed to have FeLV-related diseases were treated by ex vivo immunoadsorption with SAP-A filters or with whole SAP-A bacteria twice weekly for 10 weeks (93). An increase in serum IFN was observed immediately after treatment and was associated with improved bone marrow cytology and serological changes. Enhanced mitogen-induced blastogenic responses of lymphocytes were observed shortly after the increase in serum IFN. Both the high serum IFN titers and the mitogen responsiveness persisted throughout the tumor regressions (93). Some cats persistently infected with FeLV, reportedly had a high rate of viral clearance after ex vivo immunosorption therapy using SAP-A (94,95).

One rationale for treatment of FeLV-related disease might therefore be the use of biological response modifiers (BRMs) directed at prevention of virus-induced immunosuppression or restoration of function in immune suppressed cats. A number of BRMs, such as interferons and interleukin, are being used to positively modulate the immune system in humans with HIV-1-induced acquired immunodeficiency syndrome (AIDS) (96).

In this challenge study, oral HuIFNα treatment significantly prolonged the life of cats infected with FeLV. Though it is clear that oral administration of HuIFNα slows the development of clinical disease in cats experimentally infected with FeLV and extends the survival time of naturally infected clinically ill cats, the mechanism remains to be elucidated. Our data show that, for the most part, HuIFNα-treated cats became persistently viremic and had significant titers of FOCMA antibodies, but failed to develop any of the FeLV-related diseases. Although oral administration of IFN has not been shown to result in increased serum IFN levels (56–58), it is not known whether it affects tissue levels of IFN or other immunomodulatory molecules. Some radiolabeled HuIFNα, injected into metastatic human cancer patients, could be found transiently in the mouth, nose, and paranasal sinuses (97). Liu et al. (98) reported that injections of SAP-A intraperitoneally into FeLV-infected cats resulted in increased serum IFN and gp70-specific cytotoxic antibodies which paralleled a decrease in viremia and corrections of hematologic or cytologic abnormalities. Cats that failed to respond to SAP-A therapy also failed to develop high levels of IFN suggesting to the authors that IFN and cytotoxic antibody play important, possibly complementary roles in inducing remission of leukemia and elimination of viremia in cats (98).

In view of the poor response to treatment of cats with FeLV-related disease, more research on HuIFNα treatment may be justified. Because feline leukemia is an appropriate animal model for studying AIDS (99), confirmation of these research results and an assessment of low-dosage IFN therapy for AIDS may be desirable.

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Oral Administration of IFN-α is Superior to Subcutaneous Administration of IFN-α in the Suppression of Chronic Relapsing Experimental Autoimmune Encephalomyelitis

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We have previously demonstrated that type I IFNs administered orally (p.o.) suppress clinical relapse in murine chronic relapsing experimental autoimmune encephalomyelitis (CR-EAE), inhibit clinical attacks at doses equivalent to ineffective parenteral (s.c.) doses in acute rat EAE, and decrease the adoptive transfer of EAE. We therefore examined the optimal clinical p.o. dose of murine species-specific IFN-α for suppression of relapse attacks and compared it to s.c. administered IFN-α in a dose-response experiment in the chronic EAE model. The optimal clinically effective dose for suppression of EAE of p.o. administered murine species-specific IFN-α was 10 units and for s.c. administered was 100 units, although the optimal p.o. dose was much more clinically effective than the optimal s.c. dose. Con A- and MT-induced spleen cell proliferation was inhibited by p.o. IFN-α, as was Con A-induced IL-2 secretion, but s.c. IFN-α did not inhibit the Con A-induced proliferation in spleen cells. Oral IFN-α inhibited the mitogen-induced production of IL-2 and IFN-γ, but s.c. IFN-α increased MT-induced IFN-γ and IL-6 secretion in spleen cells and Con A-induced IL-6 and MT-induced IL-2 and IL-6 in lymph node cells. The oral route is a convenient drug delivery system that may allow the use of lower doses of cytokines and provide enhanced efficacy via unique and potent immunoregulatory circuits without generating additional inflammatory cytokines that may counteract the beneficial effects of s.c. administered type I IFNs.

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Introduction

Chronic relapsing experimental autoimmune encephalomyelitis (CR-EAE) is a T cell mediated disease that mimics the clinical disease multiple sclerosis (MS) and the associated immunological sensitization preceding clinical relapse disease. It thus provides a useful animal model for the evaluation of potential therapies for human autoimmune disease [1–3]. Previous work demonstrates that immunomodulatory cytokines can modify EAE. Intravenous natural rat interferon (10^5 units) can partially suppress acute EAE in male Lewis rats and inhibit passive hyperacute localized EAE when administered on the same day of immunogen inoculation [4, 5]. Other parenterally administered cytokines such as TGF-β can decrease clinical disease and inflammation in brain and spinal cord in EAE [6]. Parenterally administered natural human IFN-α can decrease T cell function and T cell dependent antibody production in humans [7].

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We have previously demonstrated that type I IFNs administered orally three times a week suppressed clinical relapse in murine CR-EAE [8], inhibited clinical attacks at doses equivalent to ineffective subcutaneous doses in acute rat EAE [9] and decreased the adoptive transfer of EAE by activated donor spleen cells to naive animals compared to cells from mock murine IFN fed mice [10]. Oral IFN-α inhibited IFN-γ and/or IL-2 secretion, suggesting a functional inhibition of Th1-like T helper cells in EAE, and a potential site of intervention at the level of effector T cells in MS [8–10]. Recent studies of parenterally administered human recombinant type I IFNs (hrIFN) in relapsing-remitting multiple sclerosis (RRMS) demonstrated decreased relapses [11], decreased activity on serial MRI (magnetic resonance imaging) [12], decreased spontaneous *in vitro* IFN-γ production [13], and a reduction of clinical progression, relapse rate, and gadolinium-defined inflammatory activity on MRI [14]. However, their use may be limited because 40% of IFN-β_{1b} treated patients generated neutralizing antibodies which are frequently found in patients who appear to lose both clinical benefits and MRI-defined

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responses [15]. Accordingly, we determined the most effective oral dose of the biological response modifier (BRM) murine species-specific IFN-α (mIFNα) in a dose-response experiment and compared this optimal dose to equivalent or higher subcutaneous doses of mIFN-α to examine the relative efficacy and immunological effects of oral and parenteral IFN-α in an animal model of autoimmune disease, CR-EAE.

Materials and methods

Induction of experimental autoimmune encephalomyelitis

A chronic relapsing form of EAE was induced in 7–10 week old female SJL/J mice using the method of Brown and McFarlin [16], modified by Miller [17]. Briefly, each mouse received a subcutaneous (s.c.) injection over the shaved right flank of 0.3 ml of an emulsion containing 1 mg of syngeneic mouse spinal cord homogenate (MSCH) in 0.15 ml of phosphate buffered saline and 0.03 mg of *Mycobacterium tuberculosus hominis* H37Ra (MT) (Difco Labs, Detroit, MI) in 0.15 ml of incomplete Freund's adjuvant (IFA). Seven days later, the mice received a similar injection in the left flank. Initial clinical signs of disease were seen between days 13 and 25 postimmunization, partially resolved by day 40. Clinical severity of the *relapse* attack was graded as follows by a *blinded* observer: 0=no disease; 1=minimal or mild hind limb weakness; 2=moderate hind limb weakness or mild ataxia; 3=moderate to severe hind limb weakness. Animals were scored in a blinded fashion three times a week for 15 weeks and cumulative weekly score was computed by averaging three scores per week (Monday, Wednesday, Friday) for each group of animals.

Cytokine administration

We have previously demonstrated that one and ten units of human natural IFN-α has no effect on clinical relapse disease in EAE [8]. Starting on day 40 postimmunization and after the initial clinical attack had subsided, animals were fed (p.o.) or injected subcutaneously with varying doses (0.1–1,000 units) or murine natural IFN-α (Cytoimmune mouse IFN-α, 4.0×10^5 IRU/ml, Lee Biomolecular Research, Inc., San Diego, CA, or mock murine IFN-α (Cytimmune <2 IRU/ml, Lee Biomolecular Research, Inc., San Diego, CA). (generated identically to IFN-α except cultures are mock induced)) using a 2.5 cm syringe fitted with a 20 gauge ball point needle (Thomas Scientific, Swedesboro, NJ) as previously described [8, 10]. IFN was directly delivered to the distal oesophagus, stomach and proximal small intestine (as determined experimentally by injecting Evans blue during routine feeding and subsequent sacrifice), or injected via a 27 gauge needle in the flank away from the site of inoculation three times per week (Monday, Wednesday, Friday) for 6–15 weeks. Mock IFN was used as the control since heat denatured IFN may retain some immunological activity. Mock mouse IFN-α/β control is a preparation identical to the IFN preparation (containing potentially immunoactive molecules in fibroblasts) except that the fibroblast cultures are not induced with Newcastle disease virus (according to Lee Biomolecular Research Inc., Virology and Immunology Catalog, 1989).

Lymph node cell and spleen preparation

Animals were killed 6–15 weeks after initiation of feeding. Draining inguinal nodes and spleen cells were removed and single cell suspensions were made by passage through 90 μm stainless wire meshes. Red cell lysis was performed in the spleen cell suspensions with 2 ml of ACK solution added to the pellet and the reaction was allowed to continue 5 min at room temperature.

Ex vivo T cell proliferation

Six to fifteen weeks following the onset of clinical relapse attack, mice were sacrificed, and spleen and draining inguinal lymph nodes were pooled and cultured *ex vivo* to determine Con A and *Mycobacterium tuberculosus hominis* H37Ra (MT)-specific splenocyte and lymph node proliferative responses. Antigen stimulation was carried out with MT at 10 μg/ml and mitogen stimulation with Con A at 2.5 μg/ml by incubating cells at 2×10^5 cells/well in RPMI (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (FCS) (Whittaker Bioproducts, Walkersville, MD), 1% sodium pyruvate (Gibco, Grand Island, NY), 1% glutamine (Gibco), 1% Penicillin/Streptomycin, and 50 μM 2-mercaptoethanol (standard media). The plates were incubated at 5% CO_2 and humidified at 37°C for 4 days. At that time the cells were pulsed with 2 μCi of tritiated [^{3}H] dTd and harvested 18 h later on an automated harvester. [^{3}H] dTd uptake was measured in a Beckman (liquid) scintillation counter. Cultures were run in triplicate and the results expressed as ΔCPM. Background responses were less than 500 cpm.

Cytokine analysis

Spleen and lymph node cells from mock fed, untreated or mIFN-α fed or s.c. injected animals were cultured with Con A (2.5 μg/ml) or MT at 10 μg/ml at 1×10^6 cells/ml in 75 cm^2 tissue culture flasks for 48 h in a humidified 5% CO_2/95% air incubator at 37°C. Supernatants were collected at 48 h after Con A activation and frozen at −70°C after centrifugation. Interleukin was measured using solid phase ELISA. Anti-IL-2, anti-IL-4, anti-IL-5, anti-IL-6, anti-IL-10, or anti-IFN-γ (PharMingen, San Diego, CA) antibodies were used in these assays as outlined previously [8, 10].

Statistical analysis

Statistical analysis was performed using ANOVA and Wilcoxon signed rank test.

Results

Orally administered murine species-specific IFN-α suppresses relapse at the optimal dose of 10 units three times per week for 15 weeks

We have previously shown that both 100 units or orally administered murine species-specific IFN-α/β and 100 units of hrIFN-α, but not one or 10 units human natural IFN-α, can suppress relapse attacks [8, 10]. Others have demonstrated that human IFN-α_1 has 0.1–50% of antiviral activity in L929 mouse cells compared to human WISH or HEp2 cell lines [18, 19]. Human IFN-α_2 has 1% of antiviral effect on L929 mouse cells compared to human WISH cells [20]. Since it is known that human type 1 IFN has 0.1–50% of its activity in murine systems, we determined if 0.1, 1, 10, 100 or 1,000 units of p.o. administered mIFN-α would suppress relapse attacks in a dose-response experiment. Animals (n=8/group) were immunized as described in Materials and Methods and fed three times per week with mock mIFN, 0.1, 1, 10, 100 or 1,000 units mIFN-α for 15 weeks. The mock IFN fed group on average incurred a relapse over the course of 15 weeks (Figure 1). Animals fed ten units mIFN-α showed decreasing neurological deficit from the onset of IFN feeding and maintained that effect throughout the study. Animals fed 100 units mIFN-α also showed decreasing neurological deficits although the effect was not as robust as 10 units mIFN-α. There were significant differences at each time point in clinical outcome in the mock fed versus 10 and 100 unit p.o. IFN-α fed animals. There were differences at several time points in clinical outcome in the mock fed versus 0.1, 1 and 1,000 unit p.o. mIFN-α fed animals. However, the animals fed 0.1, 1 and 1,000 units, when considered as a group tended to follow the course of the mock fed animals and were significantly worse compared to 10 units p.o. Orally administered mIFN-α demonstrated a U-shaped dose response effect. Since one unit and 1,000 units were clinically much less ineffective; and 10 units optimally and 100 units were moderately effective, this data suggests 5–50 units mIFN-α p.o. as an optimal dose in our model.

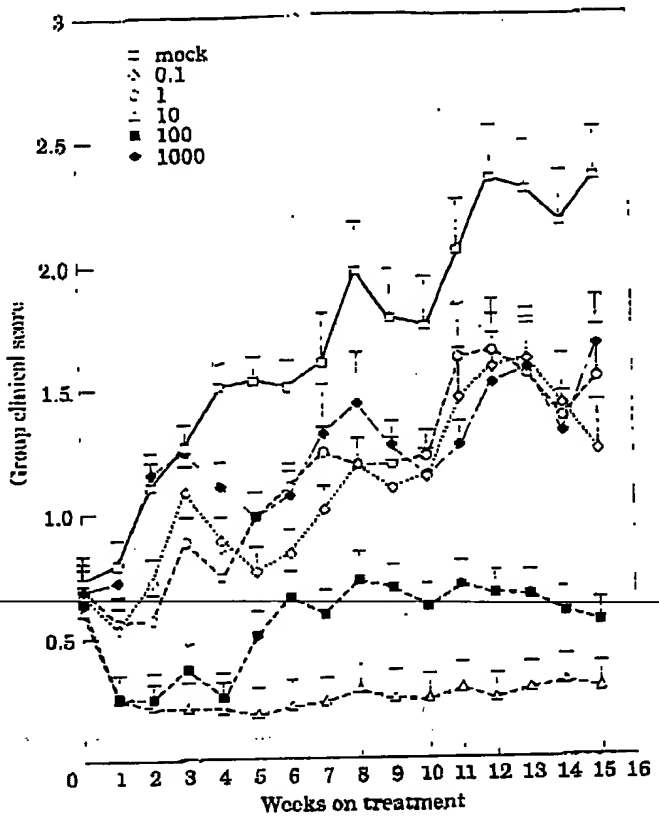


Figure 1. Oral administration of 10 units mIFN-α three times per week for 15 weeks optimally suppresses relapse attacks in murine CR-EAE. Six groups of eight SJL/J 6–8 week old female mice were immunized as described in Methods. Values represent mean weekly group clinical scores±SEM mock mIFN vs 10 units p.o. mIFN-α, weeks 1–15, $P<0.001$ by ANOVA and Wilcoxon signed rank test; mock mIFN vs 0.1, 1 and 1,000 units p.o. mIFN-α, weeks 4–6, 9–10, 12–15, $P<0.01$ by t-test; animals fed 0.1, 1, and 1,000 units were significantly worse compared to 10 units p.o. (10 units p.o. mIFN-α vs 0.1, 1, 1,000 units p.o. mIFN-α, weeks 1–15, $P<0.01$ by ANOVA and Wilcoxon signed rank test).

Orally administered IFN-α inhibits the mitogen and MT-induced proliferation in spleen cells

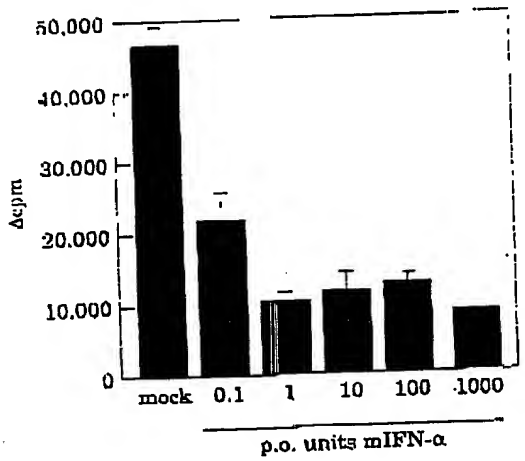
The intensity of disease in EAE has been associated with Con A proliferation of spleen cells [21]. Therefore pooled spleen cells (n=8) were stimulated from mock fed, 0.1, 1, 10, 100 and 1,000 units mIFN-α fed mice with Con A at the end of fifteen weeks. Oral mIFN-α decreased spleen cell Con A-induced proliferation significantly even at the lowest dose (0.1 unit) with nearly maximal inhibition at 1 unit (Figure 2, top). A similar response was found using a previously sensitized antigen, MT. The lowest orally administered dose had a significant effect on antigen-specific proliferation and this effect increased with increasing orally administered dosages (Figure 2, bottom).

Orally administered IFN-α inhibits the mitogen-induced production of IL-2 and IFN-γ in spleen cells

Pooled spleen cells (n=8) from mock fed, and 0.1, 1, 10, 100 and 1,000 units fed mIFN-α mice were stimulated with Con A. Low doses of p.o. mIFN-α decreased Con A-induced spleen cell IL-2 secretion at 0.1, 1, 10, and 1,000 units and IFN-γ secretion at 100 units (Table 1). There was no detectable IL-4, IL-5, IL-6, or IL-10 in these samples. These data are consistent with our previous data that demonstrated decreases in *either* IL-2 and/or IFN-γ secretion (Th1-like cytokines) in mIFN-α and hrIFN-α fed animals.

Oral administration of IFN-α is superior to equivalent or higher doses of parenteral administration in the suppression of clinical relapses in EAE

It is known that species-specific rat IFN can inhibit acute clinical attacks in rat EAE and that human type



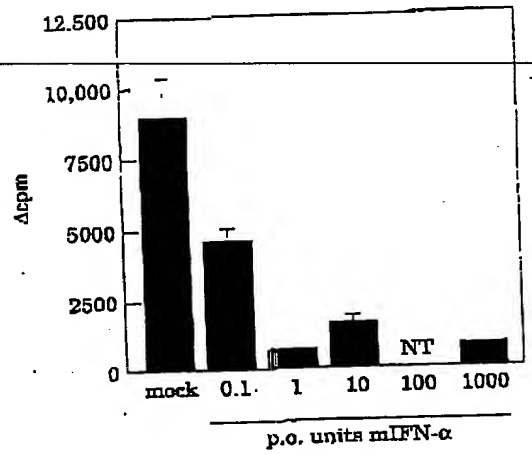


Figure 2. Oral mIFN-α decreases Con A and MT-induced proliferation in spleen cells in murine CR-EAE. Single cell suspensions from pooled spleen cells were prepared from animals (n=8/group) fed with mock mIFN or with 0.1, 1, 10, 100 or 1,000 units mIFN-α. Proliferation assays in triplicate were done with Con A (2.5 μg/ml) (top) or 10 μg/ml MT (bttom) as outlined in methods, expressed as ΔCPM±SEM. In some samples, SEM is too small to be visualized on the graph. (*Con A*: mock vs 0.1 units, P<0.05; mock vs 1, 10, 100 and 1,000 units, P<0.005; *MT*: mock vs 0.1 units, P<0.05; mock vs 1, 10 and 1,000 units, P<0.005 by Wilcoxon signed rank test). NT=not tested. Background proliferation <500 cpm.

1 IFNs have clinical effects in RRMS [9, 11, 12, 14]. We have established above that 10 units mIFN-α is the optimal oral dose in the suppression of relapse attacks. In this set of experiments, animals (n=9/group) were immunized as described in Methods and either untreated, treated with 10 s.c., or 100 s.c., 1,000 units s.c. mIFN-α or fed 10 units p.o. administered IFN-α three times per week for 6 weeks. The untreated group incurred on average a relapse over the course of 6 weeks (Figure 3; mock fed and mock s.c. injected animal controls were used but showed no difference to unfed/uninjected controls and are not shown). The 10 unit s.c. and the 1,000 unit s.c. mIFN-α groups developed steadily increasing neurological

Table 1. *Oral IFN-α inhibits Con A-induced IL-2 secretion*

spleen *Con A*	IL-2 ng/ml	IFN-γ pg/ml
mock	63.7±3.1	2,954±74
0.1 units IFN-α	30.5±3.5	2,815±103
1 units IFN-α	34.2±2.4	2,799±53
10 units IFN-α	35.8±2.4	3,302±244
100 units IFN-α	62.8±0.5	2,096±104
1,000 units IFN-α	37.2±3.1	2,828±880

Following relapse attack (week 15), mice were sacrificed, spleen cells were pooled (n=8 animals) and cultured *ex vivo* for cytokine production. Con A stimulation and interleukin assay were performed as described previously [8, 10]. Bolded values are significantly different from mock control (P<0.02, Wilcoxon signed rank test). O.D. reading in ng(pg)/ml was derived from standard curves. IL-4, IL-5, IL-6 and IL-10 were not detected in any sample tested.

deficits over time, paralleling the course of the untreated control group. However, the 100 s.c. group, after initial clinical deterioration, stabilized and was significantly better at weeks 3–6 compared to the control group and significantly better at weeks 4–6 compared to the 10 unit s.c. and 1,000 unit s.c. groups. In contrast, the 10 unit p.o. fed group improved from the initial attack and at all times showed significant differences with both the control group and all s.c. groups. This demonstrates that parenteral mIFN-α can affect EAE favorably and 100 units mIFN-α s.c. administered three times per week is optimal. However, the optimal s.c. dose was not as effective as the optimal oral dose.

Orally administered IFN-α, but not s.c. mIFN-α inhibits the Con A-induced proliferation in spleen cells

We determined if s.c. administration of mIFN-α could inhibit Con A-induced proliferation of spleen cells at the end of six weeks of treatment. Pooled spleen cells (from nine separate animals in each group) were stimulated from untreated, 10 units p.o., 10 units s.c., 100 units s.c. and 1,000 units s.c. mIFN-α treated mice with Con A. Ten units oral mIFN-α decreased Con A-induced proliferation significantly compared to the untreated group. Proliferation of 100 units s.c. mIFN-α treated mice was not significantly different from 10 units or 1,000 units s.c. mIFN-α and none of the s.c. doses were significantly decreased compared to the untreated group (Figure 4).

Oral mIFN-α inhibits the mitogen-induced production of IL-2 and IFN-γ, but s.c. mIFN-α increases Con A-induced IL-6 and MT-induced IL-2 and IL-6 production in lymph node cells

Orally administered mIFN-α decreased Con A-induced IL-2 and IFN-γ secretion in lymph node cells without having an appreciable effect on MT-induced IL-2 or IFN-γ secretion (Table 2). Ten units mIFN-α s.c. did have an immunological effect by increasing MT-induced IL-2 and IL-6 secretion in

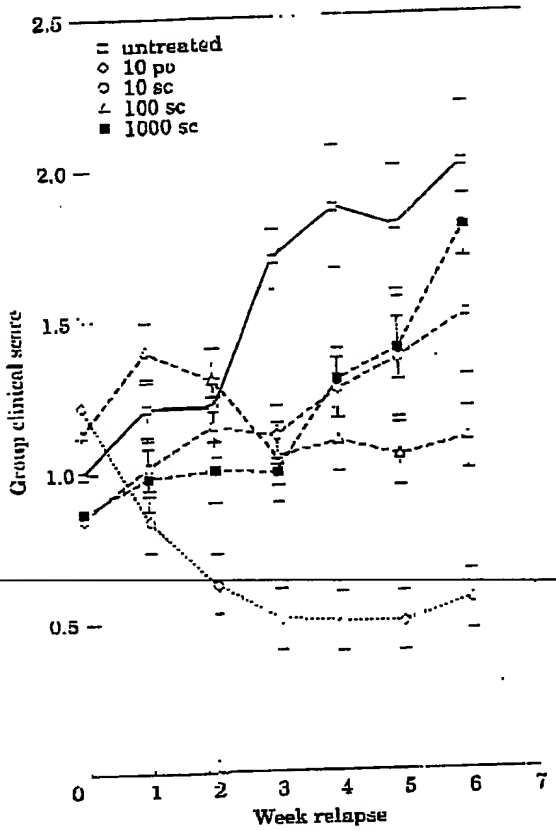


Figure 3. Ten units of oral mIFN-α is superior to equivalent or higher dose of parenteral IFN-α administered three times per week for 6 weeks in the suppression of clinical relapses in EAE. Animals (n=9/group) were immunized, followed as decribed in Figure 1 and untreated or treated with 10 units p.o., 10 units s.c., 100 units s.c. or 1,000 units s.c. mIFN-α. Values represent combined data of two separate experiments of mean weekly group clinical scores±SEM (untreated vs 10 units mIFN-α fed animals for weeks 1–6, P<0.001; vs. 100 units s.c. mIFN-α for weeks 3–6, P<0.001 by ANOVA and Wilcoxon signed rank test). Mock fed and mock s.c. injected animals as controls showed no difference to unfed/uninjected controls and are not shown.

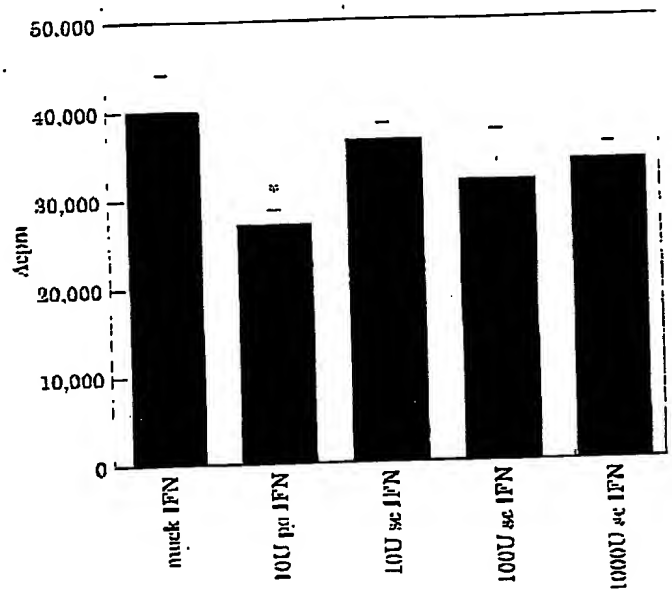


Figure 4. Oral IFN-α, but not s.c. IFN-α, inhibits Con A-induced proliferation in spleen cells in murine CR-EAE. Single cell suspensions from pooled spleen cells were prepared from animals (n=9/group) either untreated, or treated with 10 s.c., 100 s.c., 1,000 units s.c. or fed 10 units p.o. mIFN-α. Proliferation assays in triplicate were done with Con A (2.5 μg/ml) (untreated vs 10 units p.o., *P<0.05; 10 units p.o. vs. 10 s.c. and 1,000 s.c., P<0.05; untreated vs. 100 units s.c., P<0.40 Wilcoxon signed rank test). Background proliferation <500 cpm.

lymph node cells (Table 2). Increased IL-6 secretion from lymph node cells was seen only with s.c. administration of mIFN-α, whereas the oral mIFN-α group demonstrated no detectable IL-6 secretion via mitogen or MT-induced stimulation (Table 2 and data not shown for spleen). Spleen cells from s.c. treated animals demonstrated increased MT-induced IFN-γ (untreated control: 3,618 ng/ml±1,220 vs 10 units s.c.: 7,123 ng/ml±400, P<0.01) or Con A or MT induced IL-6 (Con A—100 units s.c.: 17,250 ng/ml±2,005 vs untreated or 10 units p.o.: non-detectable; MT—100 units s.c. 20,605 ng/ml±6,704 vs 10 units p.o. non-detectable; MT—1,000 units s.c.: 22,284 ng/ml±3,678 vs 10 units p.o. non-detectable). Increases of Con A or MT-induced IL-4 secretion were seen in both p.o. and s.c. administered mIFN-α splenocytes (Con A—mock: non-detectable; 10 units p.o.: 1.6 μg/ml±1.3; 10 units s.c.: 5.9 μg/ml±0.8; 100 units s.c.: non-detectable; 1,000 units s.c.: 6.0 μg/ml±4.8) (MT—mock: non-detectable; 10 units p.o.: 4.21 μg/ml±3.9; 10 units s.c.: non-detectable; 100 units s.c.: non-detectable; 1,000 units s.c.: non-detectable) and showed no correlation between route of administration and severity of clinical disease (Table 2). There was no detectable IL-5 or IL-10 in any sample tested.

Discussion

Our dose-response experiments demonstrate that 10 units of mIFN-α administered orally three times per week is the optimal dose to suppress relapses of EAE, although 100 units mIFN-α p.o. also has significant activity. Con A-induced proliferation of pooled spleen cells is inhibited by extremely low doses (0.1 units) and is maximally inhibited by as little as one unit mIFN-α p.o. This effect is maintained at 100 and 1,000 units p.o. Stimulation by sensitized antigen demonstrates a similar pattern of inhibition of proliferation at 0.1 units with maximal inhibition at one unit mIFN-α. In addition, 0.1, 1, and 10 units p.o. mIFN-α significantly decrease Con A-induced IL-2 secretion. These data suggest that both very low and very high doses of p.o. mIFN-α can have immunological effects on mitogen and antigen-induced proliferation and mitogen-induced IL-2 secretion without having significant clinical effects. We suspect the effect of very low doses of p.o. IFN is due to the IFN itself because we have successfully used recombinant human IFN-α (hrIFN-α) to inhibit acute EAE in rats, and hrIFN-α

Table 2. *Oral mIFN-α inhibits Con A-induced production of IL-2 and IFN-γ, but s.c. mIFN-α increases Con A-induced IL-6 and MT-induced IL-2 and IL-6 production in lymph node cells*

lymph node	IL-2 [ng/ml]	IL-4 [μg/ml]	IL-6 [ng/ml]	IFN-γ [ng/ml]
Con A				
mock	58.8±1.3	ND	ND	7,273±580
10U p.o. IFN	**29.1±5.6***	ND	ND	**3,736±509***
10U s.c. IFN	50.5±3.5	ND	*1,386±150*	8,992±330
100U s.c. IFN	– 57±2.2	ND	ND	9,425±645
1,000U s.c.	63.4±1.3	ND	ND	10,447±1,550
MT				
mock	22.1±1.1	ND	ND	4,811±390
10U p.o. IFN	19.7±1.4	ND	ND	4,473±140
10U s.c. IFN	*30.2±3.5**	ND	*24,877±2,109*	3,750±830
100U s.c. IFN	28.8±2.2	ND	ND	2,910±545
1,000U s.c.	15.1±1.3	ND	ND	3,662±1,233

Following relapse attack (week 6), mice were sacrificed, spleen cells or lymph node cells were pooled (n=9 animals) and cultured *ex vivo* for cytokine production. Stimulation and measurements were carried out as described previously [8, 10]. **Bolded** values are significantly decreased, *italicized* values significantly *increased* from mock control (*P<0.05, Wilcoxon signed rank test). IL-5 and IL-10 were not detected in any sample tested. ND=not detected.

does not contain other potential Newcastle virus-induced immunoactive molecules other than IFN-α that arise during manufacture of fibroblast mouse natural IFN-α [9].

The s.c. vs. p.o. dose-response experiments demonstrate that 100 units s.c. mIFN-α is the optimal parenteral dose, but this dose is not as effective as 10 units p.o. mIFN-α in suppressing relapse attacks. Ten units p.o. mIFN-α can decrease both Con A-induced IL-2 and IFN-γ secretion in lymph node cells and IL-2 secretion in spleen cells. Parenteral administered mIFN-α does not inhibit non-antigen-specific Con A-induced proliferation. However, s.c. administration of IFN-α can increase Con A or antigen-induced IL-6 secretion and increase antigen-induced IL-2, IL-6 and IFN-γ secretion. Although 100 units s.c. IFN can decrease the severity of clinical disease in EAE, the precise mechanism of action is not clear from our data. We have recently shown that IFN-βα_{1b} on-treatment CD3-mediated secretion of TNFα was significantly decreased and IL-6 secretion was significantly increased compared to pretreatment values in relapsing-remitting MS (RRMS). IFN-γ was also decreased in on-treatment cultures stimulated with anti-CD3 mAb, but these values did not reach statistical significance [22]. The mild beneficial clinical effect in EAE of s.c. IFN-α may relate to decreases in TNF-α, a cytokine that we did not measure, but this effect may be potentially counteracted by increases of another inflammatory cytokine, IL-6.

Orally administered IFN-α may exert immunological effects via inhibition of IL-2 or IFN-γ secretion. Secretion of these cytokines is characteristic of encephalitogenic Th1 T helper T cells in animals and humans [23–31]. IL-10, produced by Th2 T helper cells and a potential counterregulatory cytokine to Th1 T cells, was not detected in our system [32, 33]. In contrast, only s.c. treatment increased IL-2 and IFN-γ secretion of cells stimulated *ex vivo*, and only s.c. treated spleen or lymph node cells produced detectable qualities of IL-6 in this assay. IL-6 is an inflammatory cytokine with T cell activation properties produced by T cells and by non-T cell monocyte/macrophage cells [34–36]. If a similar process were to occur *in vivo*, the increased production of IL-6 in parenterally treated animals could counteract the potential beneficial effect of IFN-α-induced decreases in Th1-like inflammatory cytokines. Secretion of TGF-β is significantly increased via anti-CD3 mAb in RRMS patients between attacks compared to controls [37]. Therefore, other immunomodulatory cytokines, such as TNF-α and TGF-β, may have an effect on clinical disease but were not assayed during these experiments. This suggests that the superior clinical effects of p.o. administered mIFN-α compared to optimal s.c. doses in the treatment of relapses of EAE may relate to differences observed in inflammatory cytokine secretion *ex vivo* and suggests a potential critical role of the inflammatory cytokine IL-6 in mitigating the clinical efficacy of parenteral murine IFN-α, at least in the CR-EAE model.

Stanton found that low doses of recombinant hIFN-α A/D, which is highly active on mouse cells [20], or mouse IFN-α/β given orally in drinking water, protected mice from encephalitis and death from intraperitoneal (i.p.) injection of Semliki Forest Virus [38]. Importantly, this response was biphasic: neither higher nor lower doses of IFN were protective. Recent studies indicate that systemic IFN effects can be achieved with comparatively very low doses (~100–1,000 units) of natural human IFN-α [39–41]. Therefore, systemic effects may be obtained through oral administration, and the therapeutic effect may not require transit of intact IFN across the bowel. Proteins which might not survive transit through the alimentary canal may still exhibit immunomodulatory activity via the gut associated lymphoid system (GALT) in the oropharynx and beyond, via paracrine activity

42–45]. Several early studies on the pharmacokinetics of IFNs delivered by various routes reported that orally administered IFNs failed to appear in the bloodstream [44–47]. However, several investigations have shown that small but measurable amounts of IFN can be absorbed from the oral pharynx or large intestine in rats [49, 50]. There are no reports of p.o. administration of IFN peptides or the presence of IFN breakdown products in the lumen of the gut or in the bloodstream, although IFN-α amino acids 9–18 and 26–40 *in vitro* inhibit antigen receptor-stimulated proliferation or viral activity in human cells [51, 52]. More recent studies demonstrate that oral administration of low dose IFN-α in mice [53], dogs [54], monkeys [55] and humans [56] does not result in detectable levels of IFN-α in the blood, in contrast to parenteral administration, nor can its effect be blocked by circulating anti-IFN antibodies in mice [53]. The inability to detect p.o. administered IFN in blood may be due to modest spillover in a rapidly turning-over lymphatic pool [57]. The absence of increases in biological markers (β_2-microglobulin, neopterin or 2,5 OAS) after p.o. administration [56], their presence with s.c. or i.v. IFN-β [58] and the data above suggest that p.o. IFN acts through a different mechanism. The neutropenic effect of orally administered IFN can be transferred by injection of blood cells but not by serum from IFN fed animals to recipient animals or humans [53]. Activated monocytes and lymphocytes, by virtue of their circulatory ability, can potentially transfer their biological activities all over the body in the absence of circulating cytokines after contacting IFN or IFN-induced cells in the GALT [57, 59].

The immunomodulatory mechanism of orally administered IFN-α may be a decrease in precursor frequency via clonal anergy (transient IFN-induced hyporesponsiveness of encephalitogenic T cells [51, 52]) or generation of suppressor factors by T cells. Modulatory effects of Con A activated lymphocytes on the mitogen responses of normal responder cells can be abrogated by addition of anti-human leukocyte IFN serum *in vitro* [60]. Anti-human leukocyte IFN serum may prevent the production of inhibitory factors induced by IFN-α, e.g. macrophage derived suppressor factor (Mφ-SF, e.g., TGF-β) or soluble immune response suppressor (SIRS) by CD8+ T cells [61, 62]. Peripheral T cells may be required for IFN production after such mitogen stimulation [63]. Other immunoactive proteins may be important because both IFN-α and IFN-β stimulate the production of at least 12 new cellular proteins [64]. Therefore, type I IFNs may induce suppressor factors inhibiting responses to immunogenic antigens such as MT. IFN-α may be an immunomodulatory molecule produced by activated CD8+ T and other immune cells that induces suppressor factors, such as TGF-β or other cytokines, which in turn induce hyporesponsiveness to immunized antigens such as MBP and MT.

The dissociation of antiproliferative action and clinical suppression of relapse attacks with p.o. IFN-α suggests that IFN-α-induced signal transduction may vary in the various subpopulations of lymphoid cells in the gut or systemic immune system. The downregulation of IFN-α receptors and the antiproliferative action of IFN-α occur with similar dose responses: binding to a small fraction of cell surface IFN-α receptors elicits maximal antiproliferative response [65]. Variable responses to IFN-α depend on the degree and affinity of type I IFN cell surface receptors and their downregulation. The emerging image of the IFN transducing complex is of a large transmembrane macromolecule assembly with IFN, ifnar1 (IFN α/β receptor gene 1), ifnar2 (IFN α/β receptor gene 2), cytoplasmic tyrosine kinases Tyk2 and Jak1, at least one tyrosine phosphatase, and the STATs (signal transducer and activators of transcription). Since $Tyk2^-$ cells and $Jak1^-$ cells are relatively resistant to IFN-α and IFN α/β respectively, and truncation of KL (kinase function) domain within tyrosine kinases increases binding affinity of the functional receptor unit for IFNs, different affinities may be due in part to combinations of allelic forms of assembled proteins in the receptor complex [66].

We therefore hypothesize three stages of IFN-α dose effects on type I IFN receptor transducing complex present on separate T cell populations. In the first stage (low dose p.o. IFN with immunological but no clinical effects), there are IFN-α-sensitive receptors that inhibit non-antigen-specific T-cell proliferation and cytokine secretion and completely downregulate IFN-α receptors. During the next stage (clinically effective p.o. dosages), there are IFN-α-moderately sensitive receptors CD8+ suppressor T cell populations that can counterregulate CD4+ encephalitogenic T cells, or induce anergy directly on CD4+ encephalitogenic T cells. In the final stage (clinically ineffective high p.o. doses), there are IFN-α-moderately resistant receptors that inhibit function of suppressor T cells, thus disinhibiting CD4+ encephalitogenic T cell function, or reactivating previously anergic CD4+ encephalitogenic T cells.

Our studies in murine CR-EAE suggest that an ongoing immune response can be modified by orally and parenterally administered type I IFNs. The oral and parenteral administration of BRMs such as IFN-α potentially provides a continuous means of generating immunosuppression of autoreactive T cell populations. The oral route is convenient, may allow the use of lower doses, minimize side effects, and may provide enhanced efficacy via unique and potent immunoregulatory circuits without generating additional inflammatory cytokines that may counteract the beneficial effect of type I IFN. The oral route may eliminate potential therapeutic difficulties associated with neutralizing antibodies to IFN-β_{1b} in treated subjects with MS [15, 67]. Experiments are underway to determine if other cytokines such as TGF-β counterregulate IL-2, TNF-α or IFN-γ production, and if actively induced disease can be suppressed by adoptively transferred T cell subsets from untreated, 10 units p.o. fed and 100 units s.c. IFN-α treated animals.

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